FINAL REPORT

Microbiological Sampling Report

for

National Oceanic & Atmospheric Administration

Samplings Conducted on the Thirteenth Floor
of Building SSMC-4
on January 19, 2000

Interagency Agreement #: D8H00CO31200
Task: 9903

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Prepared by

US Public Health Service

Division of Federal Occupational Health

Bethesda Central Office

Executive Summary

At the request of the National Oceanic & Atmospheric Administration (NOAA), Federal Occupational Health (FOH) conducted a microbiological sampling in a cubicle area 13520 of Building SSMC-4, located at 1305 East-West Highway, Silver Spring, Maryland. The sampling was initiated due to visible fungal growth and water damage at window area of this cubicle. Sampling was conducted on January 19, 2000. Air (both Andersenâ and Zefonâ), swab, contact plate, and vacuum dust samples were collected from this cubicle and an indoor reference cubicle 13609. Air samples were also collected from outdoors.

Findings are as follows:

· Due to winter season, airborne fungal levels were low. Indoor airborne fungal and spores levels were lower than those of outdoors.

· Fungal burden on horizontal and vertical surfaces of cubicle 13520 was higher than that of 13609.

· Low fungal burden was detected from swab samples collected from surfaces of supply diffusers and return troughers in light fixture.

· *Chaetomium* dominated the sample collected from water-damaged wall surfaces at cubicle 13520.

· Fungal levels in dust samples were at 10^3 - 10^4 CFU/g of fine dust levels, except the carpet dust sample collected at the cubicle 13520 where fungal level was at 10^5 level.

· *Stachybotrys chartarum* was detected from carpet and plenum dust samples collected from cubicle 13520. Moreover, *Stachybotrys chartarum* dominated the dust sample collected from carpet below windowsill of 13520.

Recommendations are also provided.

INTRODUCTION

At the request of the National Oceanic & Atmospheric Administration (NOAA), Federal Occupational Health (FOH) conducted a microbiological sampling in a cubicle 13520 of Building SSMC-4, located at 1305 East-West Highway, Silver Spring, Maryland. The sampling was initiated due to visible fungal growth detected in window area of this cubicle. Sampling was conducted on January 19, 2000. Air (both Andersenâ and Zefonâ), swab, contact plate, and vacuum dust samples were collected from this cubicle and an indoor reference cubicle 13609. Air samples were also collected from outdoors.

EVALUATION METHODOLOGY

Air Samples

Various types of samples were collected from these cubicles on January 19, 2000. Two types of air samples were collected from each cubicle: (1) culturable method using Andersenâ N-6 samplers at a flow rate of 28.3 L/min, and (2) non-culturable method using Zefonâ Air-O-Cell cassettes at a flow rate of 15 L/min. Indoor Andersenâ air samples were collected for 3 minutes and outdoor samples were collected for both one and three minutes. Two percent (2 %) malt extract agar (MEA) and cellulose Czapek agar (CCA) was used to recover general fungi and cellulose-loving fungi, respectively. Non-culturable air samples were collected at the aforementioned sampling locations. Indoor samples were collected for ten minutes and outdoor samples were collected for both five and ten minutes. Outdoor air samples were collected near the entrance of the building. Temperature and relative humidity measurements were collected from each air sampling location by a battery operated, direct readout Hygroskopâ meter.

Swab Samples

Swab samples were collected from surfaces of each supply diffusers and return troughers in each cubicle. They were collected by wiping a known area of surface with a sterile cotton swab (Culturette^{â}) wetted with holding media. Approximately 5 in^2 area was wiped for return trougher and 4 in^2 for supply diffusers. One swab was collected from water-damaged wall surfaces at cubicle 13520. The swab was then placed directly into its holder. Each holder was labeled with an identifiable number. A total of nine swab samples were collected.

Contact Plate Samples

To determine fungal burden on horizontal and vertical surfaces of these cubicles, four contact plate samples (two from horizontal and two from vertical surfaces) were collected from each cubicle. Samples were collected from randomly selected horizontal and vertical surfaces. Sampling was conducted by pressing the MEA-filled Rodac^{â} plate against the surface of interest for five seconds. A total of 8 contact plate samples were collected.

Vacuum Dust Samples

Dust accumulated on carpeting, chairs and fabric system furniture, and the plenum were collected with a High Efficiency Particulate Air (HEPA) vacuum attached with a special “sock” device. For each carpet sample, a 3-ft by 3-ft area was vacuumed for at least five minutes. Total surface areas of 9 ft^2 were vacuumed from system furniture and chairs, and composite as one sample. Dust accumulated above the ceiling plenum was also vacuumed and composite as one sample. One carpet sample, one composite furniture sample, and one composite plenum sample were collected from each cubicle.

All samples collected were sent for next morning delivery to FOH’s Environmental Microbiology Laboratory (EML) in Philadelphia, Pennsylvania for analysis.

Laboratory Procedures

Upon receipt, all Andersen^{â} air and contact plate samples were incubated in a 25˚C incubator. Each swab sample was suspended in sterile distilled water, diluted serially, and inoculated onto agar plates. Both MEA and CCA were used for retrieving fungi. At least three dilution series were used for each sample. Each vacuum dust sample was sieved through a 250 mm sieve. The fine dust (< 250 mm) retrieved was then weighed and followed the dilution plating for fungal analysis.

All plates were incubated in a 25˚C incubator. They were examined every other day for up to 10 days to ensure the full recovery of fungi. Fungal identification was based on colony morphology, spores and conidia formation. Total fungal colonies formed on each MEA plate and *Stachybotrys chartarum* on CCA plates were counted and recorded. Fungal levels in samples were presented as colony forming units (CFUs) per measuring unit. For example, CFU/m^3 for Andersenâ air samples, CFU/in^2 for swab samples, CFU/plate for contact plate samples, and CFU/g of fine dust for vacuum dust samples.

All Zefonâ cassette samples were analyzed by the Environmental Microbiology Laboratory in Escondido, California for direct microscopic examination. Fungal spores were identified and their airborne levels were presented as spores/m^3.

RESULTS AND DISCUSSION

Temperature and Relative Humidity

Indoor temperature and relative humidity measurements ranged from 67.8°F to 69.4°F, and 16.7% – 18.7%, respectively (Table 1). Outdoors temperature reading was lower (37.6°F), but with a higher relative humidity (41.4%) (Table 1).

Microbiological Analyses Results

All laboratory analytical results from FOH's EML are presented in a laboratory report #NOAA-00-18R-B (Attachment A). Results from microscopic examination of Zefonâ cassette samples are presented in Attachment B.

Air Samples

Andersen Results

Due to winter season, airborne fungal levels were low. Outdoor airborne fungal levels were higher than those of indoors (Table 1). *Cladosporium* and Basidiomycetes dominated outdoor fungal flora. Fungi detected indoors were *Aspergillus* and *Aureobasidium.* *Stachybotrys chartarum* was not detected from these samples.

Zefon Results

Fungal spore levels were low. Fungal spores detected from both indoors and outdoors were *Cladosporium* and Basidiospores, similar to results from Andersenâ sampling. *Stachybotrys chartarum* was not detected from any sample collected.

Table 1. Temperature and relative humidity measurements and airborne fungal levels at different cubicles of the 13th floor in SSMC-4 on January 19, 2000.

Cubicles / Parameters	13609	13520	Outdoors
Temperature (°F)	67.8	69.4	37.6
Relative Humidity (%)	18.7	16.7	41.4
Airborne Fungal Levels (CFU/m^3)	24	<12	35* 71
Total Fungal Spores (Spores/m^3)	27* <7	<13* 7	53* 80

* Two samples were collected.

Swab Samples

Surfaces of Air Vents

Four of eight samples collected from surfaces of supply diffusers and return troughers in light fixtures were below the detection limits (BDL) (10 CFU/in^2 for supply diffuser and 8 CFU/in^2 for return trougher). Even samples with fungal growth showed low fungal levels (8 – 10 CFU/in^2). *Penicillium* and *Aspergillus* were recovered from these samples. *Stachybotrys chartarum* was not recovered from these samples.

Water-damaged Wall Surfaces

Chaetomium dominated the sample collected from water-damaged wall surface at cubicle 13520. *Stachybotrys chartarum* was not recovered from this sample.

Contact Plate Samples

In general, higher fungal levels were detected from cubicle 13520 than those in 13609 (Table 2). *Chaetomium*, the dominant fungal genus recovered from swab sample collected from water-damaged wall surfaces, was detected on contact plate samples collected at 13520 near water damage areas (samples #13520CP1, 13520CP2, 13520CP3). No fungi were recovered from the sample collected from the adjacent wall surface (sample #13520CP4).

Table 2. Fungal levels (CFU/plate) on horizontal and vertical surfaces of different cubicles at the 13th floor of SSMC-4, by contact plate sampling collected on January 19, 2000.

Cubicles	**13609**	**13520**
Horizontal Surfaces (CFU/plate)	7 – 8* (2**)	7 - 38 (2)
Vertical Surfaces (CFU/plate)	< 1 – 3 (2)	< 1 – 41 (2)

* Ranges. ** Total sample number.

Vacuum Dust Samples

Diverse fungal genera, such as *Alternaria, Aspergillus, Aureobasidium, Cladosporium, Epicoccum, Mucor, Paecilomyces, Penicillium, Pithomyces, Trichoderma,* Ascomycetes, and Basidiomycetes were recovered from these dust samples.

Carpet Dust

Fungal levels in these fine dust samples were at the levels of $10^4 – 10^5$ CFU/g of fine dust. Yeast, Ascomycetes, and *Cladosporium* were the dominated fungi detected from carpet dust sample collected from lobby 13609 area. However, *Stachybotrys chartarum* was the predominant fungus recovered from carpet dust sample collected from cubicle 13520 below the water-damaged windowsill (sample #13520V02) (Table 3).

Plenum Dust

Fungal levels in these fine dust samples were at the levels of 10^3 - 10^4 CFU/g of fine dust. The higher fungal level was detected from cubicle 13520 (Table 3). *Penicillium* was the predominant fungal genus recovered from these samples. *Stachybotrys chartarum* was detected from plenum dust collected from cubicle 13520.

Furniture Dust

Fungal levels in furniture dust collected from lobby sofa and panel adjacent to cubicle 13520 were at the levels of 10^3 - 10^4 CFU/g of fine dust. Predominant fungi recovered from these dust samples were *Aureobasidium, Cladosporium, Alternaria,* general outdoor fungi. *Stachybotrys chartarum* was not detected.

FINDINGS

· Due to winter season, airborne fungal levels were low. Indoor airborne fungal and spores levels were lower than those of outdoors.

· Fungal burden on horizontal and vertical surfaces of cubicle 13520 was higher than that of 13609.

· Low fungal burden was detected from swab samples collected from surfaces of supply diffusers and return troughers in light fixture.

· *Chaetomium* dominated the sample collected from water-damaged wall surfaces at cubicle 13520

· Fungal levels in dust samples were at 10^3 - 10^4 CFU/g of fine dust levels, except the carpet dust sample collected at the cubicle 13520 where fungal level was at 10^5 CFU/g of fine dust level.

· *Stachybotrys chartarum* was detected from carpet and plenum dust samples collected from cubicle 13520. Moreover, *Stachybotrys chartarum* dominated the dust sample collected from carpet below windowsill of 13520.

CONCLUSIONS

Results from this microbiological sampling indicated that fungal contamination occurred at windowsill area of cubicle 13520. The contamination was detected on carpeting and above ceiling plenum with elevated fungal levels and alteration of fungal taxa.

Table 3. Total fungal levels (CFU/g of fine dust) and dominant fungal taxa in fine dust collected from carpet, plenum, and furniture of cubicles 13609 and 13520 by vacuum dust sampling, collected on January 19, 2000.

	Fungal Levels	Predominant Fungi
Carpet		
Lobby 13609	22,400 (-*)	Yeast > Asco > Cla**
Below windowsills of cubicle 13520	601,980 (+)	SC > Pen > Asp
Plenum		
Above lobby 13609	5,940 (-)	Pen > Asp > Alt
Above cubicle 13520	34,800 (+)	Pen > SC > Asp
Furniture		
Sofa in lobby 13609	14,000 (-)	Aur > Alt = Epi
Panel adjacent to cubicle 13520	2,600 (-)	Cla > Asp > Aur

* +: *Stachybotrys chartarum* was detected on MEA and/or CCA plates.

-: *Stachybotrys chartarum* was not detected on MEA and CCA plates.

** Asco: Ascomycetes, Cla: *Cladosporium*, SC: *Stachybotrys chartarum*,

Pen: *Penicillium*, Asp: *Aspergillus*, Alt: *Alternaria*, Aur: *Aureobasidium*,

Epi: *Epicoccum.*

RECOMMENDATIONS

· Seal up the water-damaged and visible fungal growth areas of cubicle 13520 to prevent any release of fungal spores.

· Thoroughly HEPA vacuum the carpeting and ceiling plenum at cubicle 13520 area, after-hours, to remove existing fungal spores.

· Conduct a visual inspection of cubicle 13520 area to determine the extent of contamination in order to abate the area according to guidelines provided by the American Conference of Governmental Industrial Hygienists (ACGIH) and New York City Department of Health.

· Investigate cubicle 13520 area to determine the cause of water intrusion and fix the problem permanently.

· Conduct any above ceiling plenum work after hours. Thoroughly HEPA vacuum the surrounding areas afterwards.

· Implement an emergency water intrusion protocol for this building to adequately manage any unexpected water intrusion in order to prevent fungal proliferation.

ATTACHMENT A

Microbiological laboratory report for samples collected

from thirteenth floor of SSMC-4, on January 19, 2000.

ATTACHMENT B

Results from microscopic examination of Zefon air samples collected

from thirteenth floor of SSMC-4, on January 19, 2000.

USPHS DFOH ENVIRONMENTAL MICROBIOLOGY LABORATORY, PHILADELPHIA, PA

LABORATORY REPORT #NOAA-00-18R-B

Client agency: National Oceanic and Atmospheric Administration, Silver Spring, MD

POIS#/task #: D8H00CO31200 / 9903

Sampling date: 1/19/00

Dates of inoculation: 1/19/00 (airs and contact plates) 1/20/00 (wipes) and 1/21/00 (dust)

General location: SSMC-4, Silver Spring, MD

Specific location: 13th floor

Sampling techniques: Air (Andersen N-6 sampler), contact plate, wipe, and vacuum dust samplings

Medium used: Malt extract agar (MEA) and Cellulose Czapek agar (CCA) for fungi

Samples submitted by: J. Sobelman

Date characterization completed: 1/31/00

(A) Air samples on MEA and CCA plates

Sample ID	Sampling Location	Air Volume (L)	Fungi on MEA @ 25º C	Presence of *Stachybotrys chartarum**** on CCA @ 25º C
OAMA1, CA1	Outside bldg. 4	84.9	1. *Cladosporium* (2*) 2. *Alternaria* (1) 3. *Aspergillus sp.* (1) 4. Basidiomycetes (2) CFU/m^3 = 71	No
OAMA2, CA2	Outside bldg. 4	28.3	1. *Cladosporium* (1) CFU/m^3 = 35	No
Field blank	Blank	NA#	No fungal growth	No
13609MA1, CA1	13th floor, lobby 13609	84.9	1. *Aspergillus sp.* (1) 2. *Aureobasidium* (1) CFU/m^3 = 24	No
13520MA1, CA1	13th floor, adjacent to 13520, hallway	84.9	No fungal growth CFU/m^3 < 12	No
SB	Shipping blank	NA	No fungal growth	No

(B) Contact plate samples on MEA plates

Sample ID	Sampling Location	Fungi detected on MEA @ 25º C
13609CP1	13th floor, lobby 13609, end table with plant	1. *Aureobasidium* (2) 2. *Penicillium* (2) 3. *Aspergillus sp.* (1) 4. *Cladosporium* (1) 5. Basidiomycetes (1) **CFU/plate = 7**
13609CP2	13th floor, lobby 13609, end table with lamp	1. *Aspergillus sp.* (3) 2. *Alternaria* (1) 3. *Aspergillus niger*** (1) 4. *Epicoccum* (1) 5. *Mucor* (1) 6. *Penicillium* (1) **CFU/plate = 8**
13609CP3	13th floor, lobby 13609, wall near 13604	No fungal growth CFU/plate < 1
13609CP4	13th floor, lobby 13609, adjacent to 13604, wall behind sofa	1. *Aureobasidium* (1) 2. *Cladosporium* (1) 3. *Rhizopus* (1) **CFU/plate = 3**

13520CP1	13th floor, cube 13520, wall @ water damage	1. *Mucor* (30) 2. *Chaetomium* (5) 3. *Cladosporium* (3) 4. *Penicillium* (2) 5. *Alternaria* (1) **CFU/plate = 41**

Sample ID	Sampling Location	Fungi detected on MEA @ 25º C
13520CP2	13th floor, cube 13520, windowsill near damage	1. *Mucor* (25) 2. *Chaetomium* (7) 3. *Penicillium* (3) 4. *Cladosporium* (2) 5. *Alternaria* (1) **CFU/plate = 38**
13520CP3	13th floor, cube 13520, file cabinet in front of window	1. *Chaetomium* (4) 2. *Nigrospora* (2) 3. *Penicillium* (1) **CFU/plate = 7**
13520CP4	13th floor, cube 13520, on adjacent wall	No fungal growth CFU/plate < 1
SB	Shipping blank	No fungal growth

(C) Wipe samples on MEA and CCA plates

FOH ID	Sample ID	Sampling Location	Area (in^2)	Dilution factor	Fungi on MEA @ 25°C	Presence of ***Stachybotrys chartarum**** on CCA @ 25º C

4	13609S1	13th floor, lobby 13609, supply	4	40X-MEA 10X-CCA	1. *Penicillium* (1) CFU/in^2 = 10	No
5	13609S2	13th floor, lobby 13609, supply	4	40X-MEA 10X-CCA	No fungal growth CFU/in^2 < 10	No
6	13609R1	13th floor, lobby 13609, return	5	40X-MEA 10X-CCA	No fungal growth CFU/in^2 < 8	No
7	13609R2	13th floor, lobby 13609, return	5	40X-MEA 10X-CCA	1. *Aspergillus sp.* (1) CFU/in^2 = 8	No

FOH ID	Sample ID	Sampling Location	Area (in^2)	Dilution factor	Fungi on MEA @ 25°C	Presence of *Stachybotrys chartarum**** on CCA @ 25º C
8	13609R3	13th floor, lobby 13609, return	5	40X-MEA 10X-CCA	1. *Paecilomyces* (1) CFU/in^2 = 8	No
9	13520S1	13th floor, cube 13520, supply 1	4	40X-MEA 10X-CCA	1. *Penicillium* (1) CFU/in^2 = 10	No
10	13520S2	13th floor, cube 13520, supply 2	4	40X-MEA 10X-CCA	No fungal growth CFU/in^2 < 10	No
11	13520R1	13th floor, cube 13520, return 1	5	40X-MEA 10X-CCA	No fungal growth CFU/in^2 < 8	No
12	13520W1	13th floor, cube 13520, wall @ water damage	NA	4,000X-MEA 10X-CCA	1. *Chaetomium* (36) 2. *Penicillium* (1) CFU/swab = 1.5 x 10^5	No

(D) Vacuum dust samples on MEA and CCA plates

FOH ID	Sample ID	Sampling Location	Weight (g)	Dilution factor	Fungi on MEA @ 25°C	Presence of *Stachybotrys chartarum**** on CCA @ 25º C
V04	13609V01	13th floor, lobby 13609, sofas	0.100##	400X-MEA 10X-CCA	**1. *Aureobasidium* (2)** 2. *Alternaria* (1) 3. *Epicoccum* (1) 4. *Penicillium* (1) 5. Ascomycetes (1) 6. Basidiomycetes (1) CFU/g = 1.4×10^4	No

FOH ID	Sample ID	Sampling Location	Weight (g)	Dilution factor	Fungi on MEA @ 25°C	Presence of *Stachybotrys chartarum**** on CCA @ 25º C
V05	13609V02	13th floor, lobby 13609, carpet	0.100	40X-MEA 10X-CCA	**1. *Cladosporium* (4)** 2. *Aureobasidium* (3) 3. *Penicillium* (2) 4. *Aspergillus sp.* (1) 5. Ascomycetes (15) 6. yeast (31) CFU/g = 2.2×10^4	No

V06	13609V03	13th floor, lobby 13609, above ceiling plenum	0.101	40X-MEA 10X-CCA	**1. *Penicillium* (6)** 2. *Alternaria* (3) 3. *Aspergillus sp.* (3) 4. *Aspergillus niger*** (2) 5. *Mucor* (1) **CFU/g = 5,940**	No
V08	13520V03	13th floor, cube 13520, above ceiling plenum	0.100	40X-MEA 40X-CCA	**1. *Penicillium* (49)** 2. *Stachybotrys chartarum**** (29) 3. *Aspergillus sp.* (3) 4. *Aspergillus flavus**** (2) 5. *Cladosporium* (2) 6. *Alternaria* (1) 7. *Aspergillus niger*** (1) CFU/g = 3.5×10^4	Yes (3) CFU/g = 1,200

FOH ID	Sample ID	Sampling Location	Weight (g)	Dilution factor	Fungi on MEA @ 25°C	Presence of *Stachybotrys chartarum**** on CCA @ 25º C

V09	13520V01	13th floor, cube 13520, back of system furniture from adjacent cubes	0.100##	40X-MEA 10X-CCA	**1. *Cladosporium* (4)** 2. *Aspergillus sp.* (3) 3. *Aureobasidium* (2) 4. *Alternaria* (1) 5. *Aspergillus niger*** (1) 6. *Penicillium* (1) 7. *Trichoderma* (1) CFU/g = 2,600	No
V10	13520V02	13th floor, cube 13520, carpet below windowsill	0.101	400X-MEA 40X-CCA	**1. *Stachybotrys chartarum**** (129)** **2. *Penicillium* (16)** 3. *Aspergillus sp.* (7) CFU/g = 6.0 x 10^5	Yes (TNTC)§ CFU/g > 1.6 x 10^5

* Colony counts.

** Opportunistic fungi.

*** Toxigenic fungi.

Not applicable.

5ml of sterilized distilled water were added instead of 10ml.

§TNTC Too numerous to count.

Characterization completed by: _____

Ling-Ling Hung, Ph.D. Microbiologist

Quality control checked by: _____ (initials)